THE ABSORPTION AND TRANSLOCATION OF PHOSPHORUS AND IRON IN RICE PLANTS

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Introduction

It has been generally recognized that iron is a non-mobile nutrient and its absorption and translocation in plant tissues are affected by various factors. Recently Rediske and Biddulph (1) and the latter (2) have indicated in their comprehensive studies on the absorption and translocation of iron using red Kidney beans, that the iron absorption increases as the iron concentration or the hydrogen ion concentration of the nutrient solution increases, and the high phosphorus concentration in the solution retards the uptake of iron. Also, concernig the rate of redistribution of injected iron, they found that the iron concentration of the tissues was the primary factor in determining the mobility of iron, and that when the tissue concentration was lowest its mobility was greatest.

The absorption and translocation of iron are also influenced by manganese (3, 4). Using radioiron, Sideris (5) has shown that in cultures with manganese most iron absorbed was deposited in the roots and the transfer of iron from roots to leaves was low. Sideris and Pierre (6) have found manganese toxicity symptoms to be less pronounced at a high iron level than at low concentrations indicating an antagonism between iron and manganese.

On the contrary iron influences the uptake of phosphorus, as described by Biddulph (2), and the absorption of phosphorus is highest from solutions which are low in iron. Accordingly, the absorption and translocation of phosphorus are considered to be affected by manganese having relation to iron.

To see the effects of the iron and manganese concentrations in plant tissues on the absorption of iron and phosphorus, rice plants were cultured, prior to the exterimental period, in solutions of various iron and manganese levels, then the rates of absorption and translocation of phosphorus and iron were determined using radioisotopes .

Materials and methods

Seedlings of rice plant (*Norin No.* 16), which had been grown in sand beds, were cultured in 3-liter porcelain pots by the technique described below. Each pot had its enameled wooden cover plate pierced with 12 holes of 1.5 cm in diameter, and 4 seedlings were transplanted to each hole with the aid of cotton. Then the pots were filled with the nutrient solutions of the following salts and concentrations: NH_4NO_3 0.001 M, KH_2PO_4 0.0002 M, KCl 0.0003 M, $MgSO_4$ 0.0002 M, $CaCl_2$ 0.0001 M, and manganese chloride and ferric citrate as indicated for each treatment. The concentrations of iron used were 0.0 (not added), 0.1, 1.0, and 10.0 p.p.m. Fe_2O_3 at a level of 0.1 p.p.m. Mn_2O_3, in addition as high manganese treatments 10.0 p.p.m. Mn_2O_3 were added to the pots of 0.0 and 1.0 p.p.m. Fe_2O_3. All culture solutions were prepared with distilled water. To simplify the description, these six treatments will hereafter be referred to as Fe-0.0, Fe-O.O-hMn, etc.

The pH of the culture solutions was adjusted to 5.0 and the solutions were renewed every week. After four weeks of the intial period of growth these cultures were divided into two sets. One set of cultures were added with radiophosphorus (P^{32}) at the rate of 10 μc per pot, and to another radioiron was supplied in the form of ferric chloride at the rate of 4 μc per pot. In all culture solutions the concentrations of Fe_2O_3 and Mn_2O_3 were made to 1.0 and 0.1 p.p.m. respectively, and as the iron source $FeCl_3$ was used.

At the end of the absorption period, a weke for P^{32} and two weeks for radioiron, the plants were harvested. After the roots were washed carefully in running tap water, these harvested plants were divided into leaves and roots. Further, the leaves were separated into lower, upper, and new leaves: namely from the base, the leaves from the first to the fourth, the 5 th and the 6th leaves, and the 7th and the 8th leaves respectively. The separated materials were then dried at 70°C.

The amounts of P^{32} contained in each plant tissue were determined as follows. The plant materials were put into porcelain crucibles cautiously, and ashed in a muffle furnace at 550°C for one hour. After cooling, each ash was dissolved with the addition of 5 ml of N-HCl and an aliquot of the supernatant of the resulting solution was evaporated to dryness in a glass disc. Then the disc was placed in a Lauritsen electroscope and the radioactivity was estimated.

To prepare the samples for the determination of radioiron the following technique was used. The plant materals were placed in 50-ml Kjeldahl flasks carefully and digested with sulfric, nitric, and perchloric acids. (7) After cooling the contents of the flasks were transfered to 50-ml beakers. As the amounts of iron contained in the leaves were very small, 2 mg of inert iron in the form of $FeCl_3$ and more phosphate than equivalent to it were added to each beaker as

carrier to precipitate radioiron quantitatively and to avoid missing it in the succeeding procedures. After each solution was almost neutralized with NH_4OH, it was added with 10 ml of N ammonium acetate buffer of pH 4.0 and heated on a boiling water bath for two hours to complete the precipitation of ferric phosphate. The precipitate was filtered, washed with 5 per cent NH_4NO_3 solution, dried, and put into a crucible with the filter paper, then ignited in a muffle at 550°C. The ignited residue in the crucible was carefully transfered to a stainless steel counting disc and spreaded evenly with a glass rod. Then the disc was placed in a Geiger Müller counter and counted.

The radioiron used was a mixture of Fe^{55} and Fe^{59} at a proportion of about 2 to 1. But it was about two years later being assayed when the radioiron was employed in the experiment, so that the isotope Fe^{59} of shorter half-life (47 days) should have decayed away to a negligible quantity at the experimental period. Therefore, it was the isotope Fe^{55} of longer half-life (4 years) which was used.

Results and discussion

At the end of the preliminary culture period the growth of the rice plants was best both in the Fe-1.0 and in the Fe-10.0 cultures, while it became worse as the iron concentration in the culture solution became lower, as indicated in Table 1. Moreover, the high manganese teratments depressed the growth especially at the lower iron level.

Table 1. Growth indexes of rice plants at the end of the preliminary culture pericd in various solutions different in the concentrations of iron and manganese.

	Fe-0.0	Fe-0.0 -hMn	Fe-0.1	Fe-1.0	Fe-1.0 -hMn	Fe-10.0
Length of shoot, cm	30.6	26.5	37.9	43.2	40.9	44.3
Length of roots, cm	12.6	11.3	13.7	17.3	14.0	15.0
Dry wt., mg/4 plants						
Upper leaves	132	85	296	396	307	396
Lower "	120	147	172	184	155	176
Total of leaves	252	232	468	580	462	572
Roots	131	96	268	252	178	254

In the Fe-0.0 cultures their upper leaves showed symptoms of iron deficiency chlorosis clearly, but their lower leaves did not show it so distinctly, because the seedlings used might have absorbed small quantities of iron during their growth period in the sand beds supplied with tap water. All the leaves, particularly the upper, which received the Fe-0.0-hMn treatment presented severe symptoms induced by the toxicity of manganese in addition to the lack of iron. These symptoms of manganese toxicity were entirely different from

those of iron dificiency and appeared rather necrotic as in the case of the observations by Morries and Pierre in their experiments on several plants (8). As described later these necrotic leaves could not fully recover from their injury, although appreciable amounts of iron were absorbed during the following experimental period when the manganese concentration of nutrient solutions were lowered and iron was supplied. On the contrary, the plants which received the high manganese treatment at a level of 1.0 p.p.m. Fe_2O_3, showed no distinct symptoms of injury, though their growth was somewhat reduced. Morris and Pierre (6) also described in their experiments with *Lespedeza*, that manganese toxicity symptoms were found to be much less pronounced at a high iron level than at low concentrations indicating an antagonism between these two nutrients. When seedlings were cultured in solutions containing 0.1 p.p.m. Fe_2O_3, no symptom of iron deficiency chlorosis was recognized, though the iron supplement seemed to be insufficient to attain their maximum growth. Then these plants were supplied with radiocative phosphorus or iron as tracter, and allowed to absorb as already described.

The absorption of phosphorus

During the one-week experimental period using P^{32} the Fe-0.0 plants grew a healthy new leaf (the 7th) and the symptoms of iron deficiency chlorosis began to recover in the upper portions of them, being supplied with iron. However, the plants of the Fe-0.0-hMn culture could hardly recover from the injury which had been induced by the excess of manganese during the intial period of growth, and their dry weight increased only slightly, although the manganese levels

Table 2. Amounts of radiophosphorus absorbed in a week by the plants previously cultured in solutions of various iron and manganese levels. The values are experiessed as mg per 4 plants.

	Fe-0.0		Fe-0.0-hMn		Fe-0.1	
	Dry wt.	Absorbed $P^*_2O_5$	Dry wt.	Absorbed $P^*_2O_5$	Dry wt.	Absorbed $P^*_2O_5$
New leaves	65	0.252	—	—	159	1.030
Upper 〃	157	0.380	149	0.274	306	1.216
Lower 〃	135	0.192	127	0.119	127	0.212
Total of leaves	357	0.824	276	0.393	592	2.458
Roots	154	0.624	135	0.508	248	1.180

	Fe-1.0		Fe-1.0-hMn		Fe-10.0	
	Dry wt.	Absorbed $P^*_2O_5$	Dry wt.	Absorbed $P^*_2O_5$	Dry wt.	Absorbed $P^*_2O_5$
New leaves	132	0.546	99	0.567	158	0.719
Upper 〃	370	1.156	324	1 204	376	1.242
Lower 〃	159	0.264	140	0.175	154	0.244
Total of leaves	661	1.966	563	1.945	688	2.205
Roots	274	1.054	214	1.000	294	1.270

in the culture solutions were lowered to 0.1 p.p.m. Mn_2O_3 (Tables 1, 2). While in the other plants the 8th leaf began to develop.

The amounts of radiophorus absorbed by these plants are shown in Table 2, and Figure 1 indicates them on a basis of g dry tissue. Biddulph (2) found that the absorption of phosphorus is high from solutions which are low in iron, and on a concentration basis it is highest from solutions at the lowest iron concentrations. In the present experiment, though the iron concentrations in nutrient solutions were varied while the preliminary culture, during the experimental period iron was supplied at a uniform level of 1.0 p.p.m. Fe_2O_3. Thus the highest absorption of P^{32} was seen with the plants cultured previously

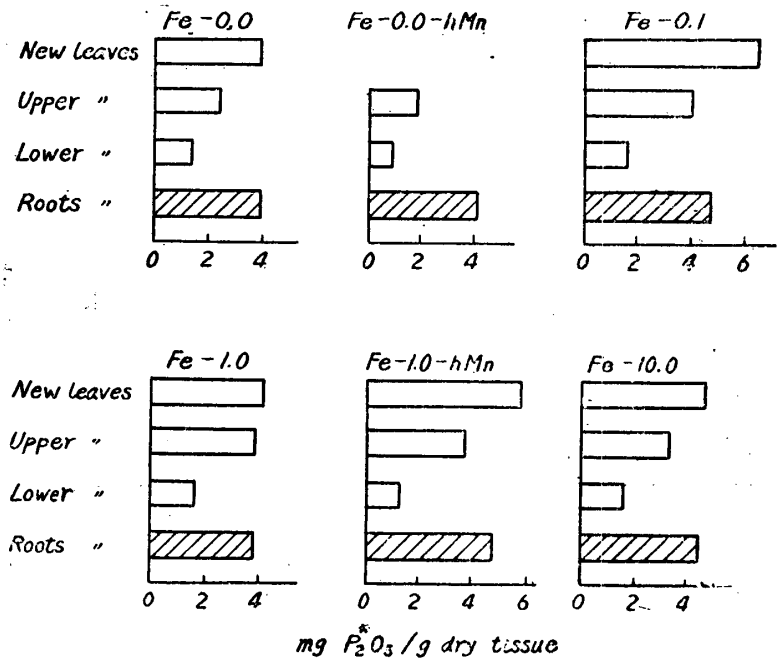

Fig. 1 The effects of the iron and manganese concentrations of nutrient solutions on the succeeding uptake of phosphorus (P*). The absorption period was a week. The values represent mg $P^*_2O_5$ absorbed per g dry tissue.

at the level of 0.1 p.p.m. Fe_2O_3.

Owing to their depressed original growth, the plants of the Fe-0.0 culture absorbed less phosphorus. This was especially evident with the Fe-0.0-hMn plants depositing much of the absorbed P^{32} in the roots. The Fe-0.1 plants showed not only the most active absorption and translocation of P^{32}, but attained extensive growth during the absorption period uptaking much amounts of iron as described later.

The absorption of P^{32} by the plants which had grown in solutions of higher iron concentrations, Fe-1.0 and Fe-10.0, was also considerably high, but the rate of the translocation of it to the new leaves was smaller compared with the

Fe-0.1 plants suggesting the antagonic effects of iron on the transfer of phosphorus in plant tissues. As shown in Figure 1, the new leaves of the Fe-1.0-hMn plants accumulated fairly higher concentrations of P^{32} than those of the Fe-1.0, when the manganese level in solutions was lowered. This might be possibly due to their low concentrations of iron originally contained as discussed later, but this can not be confirmed here since the iron contents in the tissues were not determined.

The absorption of iron

After the 2-week absorption period the plant tissues were analysed for their contents of radioiron, and the results obtained are indicated in Table 3 and Figure 2. The total amounts of radioiron transfered into the leaves were largest with the plants of the Fe-0.1 culture and smallest with those of the Fe-0.0-hMn and Fe-10.0 cultures.

Table 3. Amounts of radioiron absorbed during the 2-week experimental period by the plants previously cultured in solutions of various iron and manganese levels. The values are based on 4 plants.

	Fe-0.0		Fe-0.0-hMn		Fe-0.1	
	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg
New leaves	273	63	37	8	630	117
Upper 〃	198	27	120	10	390	42
Lower 〃	117	9	99	19	128	27
Total of leaves	588	99	256	37	1148	186
Roots	224	226	101	141	453	745

	Fe-1.0		Fe-1.0-hMn		Fe-10.0	
	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg	Dry wt. mg	Absorbed $Fe^*_2O_3$,μg
New leaves	484	49	472	50	536	23
Upper 〃	345	17	291	35	413	7
Lower 〃	109	11	114	30	130	13
Total of leaves	938	77	877	115	1079	43
Roots	429	437	369	424	496	840

By the supplements of iron the Fe-0.0 plants were cured from the symptoms of iron deficiency chlorosis almost completely. Their uptake of radioiron was very active and about 30 per cent of the total absorbed iron were transfered from the roots to the leaves (Table 3). As can be seen from Figure 2, the new leaves of the Fe-0.0 plants had especially high concentrations of radioiron, indicating that the plants lacking in iron absorbed the supplied iron rapidly and translocated it into the growing parts preferentially, so that the recovery from chlorotic appearance began in the new leaves first of all. On the contrary, the absorption of radioiron by the Fe-10.0 plants was most inactive owing to the high iron contents in their roots and only 5 per cent of the total absorbed iron

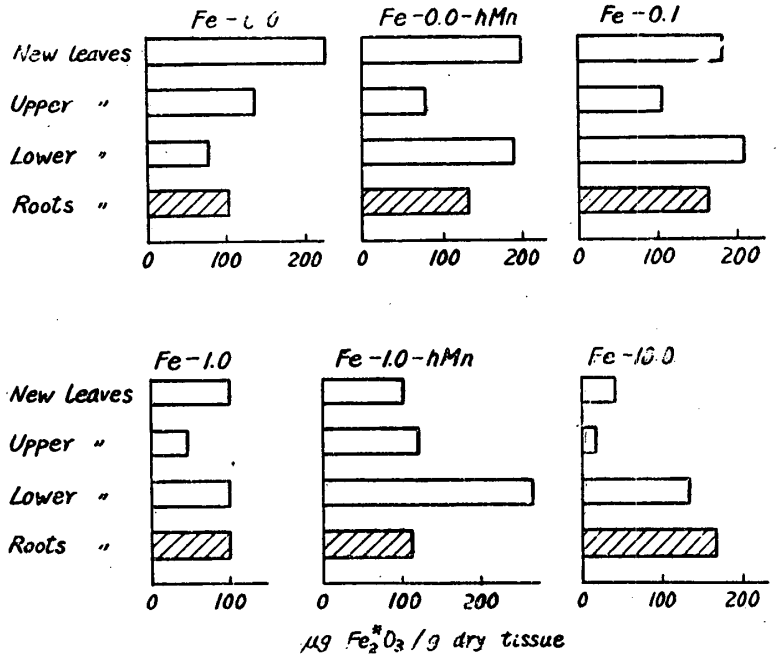

Fig. 2 The effects of the iron and manganese concentrations of nutrient solutions on the succeeding uptake of iron (Fe*). The absorption period was 2 weeks. The values represent μg $Fe^*_2O_3$ absorbed per g dry tissue. Multiply those for roots by ten.

were transfered to the leaves from the roots. Moreover, the rate of translocation of absorbed iron to the upper leaves was very small and the lower leaves had higher concentrations of it. It is clear that the iron concentration of tissue affects the absorption and translocation of iron greatly, and when it is lower the rate of translocation of iron is higher. Using red Kidney beans, Rediske and Biddulph have recognized the primary effects of the tissue concentrations of iron on the mobility of injected iron (1).

Although no symptom of chlorosis appeared in the plants of the Fe-0.1 culture, they might have been somewhat deficient in iron, so the total amounts of the absorbed radioiron was largest and the rate of its translocation was also high. Since their absorption of phosphorus during the experimental period was also high as described above, these plants showed excellent growth and their dry weight became larger than those of the other plants. As shown in Figure 2, not only the new leaves but also the lower leaves had high contents of radioiron. It seems that the iron contents of the new and upper leaves would attain certain levels during the absorption period and thereafter the absorbed iron accumulated in the lower leaves. As the plants of the Fe-1.0 culture had absorbed enough iron during the initial period of growth, the rates of absorption and translocation of radioiron in them were reduced to about half of those in the Fe-0.1 plants.

In the plants of the Fe-0.0-hMn culture not only their growth was worse, but

also, even when the manganese levels in the solutions were lowered, the recovery from the injury was slower and more incomplete compared with the Fe-0.0 plants. Their new leaves which developed during the experimental period, were green and contained large amounts of radioiron. Although the lower leaves had considerably high concentrations of iron, the symptoms of chlorosis were not cured, indicating that their chlorotic symptoms were induced by the toxicity of manganese and had become too severe to be cured by the absorption of iron.

When expressed in dry weight basis, the amounts of radioiron uptaken by the Fe-1.0-hMn plants were larger than those of the Fe-1.0 plants, but the absorbed iron accumulated much in the lower leaves. Bennett (4) described that the absorption of iron is antagonized by manganese and the excess of it depresses the transfer of iron in plants. Also, Sideris (5) has shown that most iron absorbed from solutions is deposited in the roots in cultures with manganese and the translocation of iron into leaves is lowered. So it may be supposed that in the Fe-1.0-hMn plants the absorption of iron was suppressed by the high manganese level of culture solutions during the initial period of growth and their contents of iron were lower than those of the Fe-1.0 plants. Therefore, when the manganese concentrations in the solutions were decreased, their absorption of iron might become higher but most of the absorbed iron would deposit in the lower parts of the plants affected by the high manganese concentrations of the tissues.

Summary

To investigate the effects of the iron and manganese concentrations of the tissues on the absorption and translocation of phosphorus and iron, rice plants were grown in nutrient solutions with varying amounts of iron and manganese for four weeks and after this initial period of growth the plants were supplied with radioisotopes of phosphorus and iron and their uptake of these nutriments were examined.

The absorption and translocation of phosphorus is highest in the plants previously grown in solutions of a lower iron concentration (0.1 p.p.m. Fe_2O_3). The plants grown with higher iron levels absorb appreciable amounts of phosphorus though less than the low iron plants, but the translocation of it is suppressed suggesting the antagonic effects of iron on the transfer of phosphorus in the tissues. Due to their inferior growth the chlorotic plants cultured without iron decreased their uptake of phosphorus considerably regardless of their having the lowest iron contents. The excess amounts of manganese in the solutions depress the growth of rice plants especially of the lowest iron plants, and these latter plants which have represented severe symptoms of manganese toxicity

can hardly uptake the supplied phosphorus. While at a higher iron concentration of the solution (1.0 p.p.m.) manganese toxicity has not appeared and the plants grown in this solution uptake later as much phosphorus as the low iron plants when the manganese concentration of the solution is lowered.

The uptake of iron is greatly influenced by the tissue concentrations of it. The lower the tissue concentration is, the higher is the absorption and translocation of iron and vice versa. The iron deficient plants absorb the supplied iron rapidly and transfer much of the absorbed iron to the growing leaves, so the symptoms of iron deficiency chlorosis are cured from the new leaves in a few days. Although no distinct appearance of chlorosis has been shown in the plants grown with a lower iron concentration (0.1 p.p.m.), these plants seem to have been nearly short in iron. Therefore, they uptake iron actively and accumulate it first in the growing leaves then in the lower leaves. In the highest iron plants not only the rates of absorption and translocation of iron decrease remarkably but also most of the iron transfered to the leaves deposit in the lower parts. The plants having grown with the excess amounts of manganese absorb large quantities of iron provided the manganese levels in the solutions are reduced, but the high concentrations of manganese in the tissues tend to antagonize the translocation of iron.

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